



SEQUENCE LISTING

<110> Genencor International, Inc.
Poulose, Ayrookaran J.

<120> Multiply-Substituted Protease Variants

<130> GC717-2-PCT

<140> PCT/US03/01447

<141> 2003-01-16

<150> US 60/350,222

<151> 2002-01-16

<160> 10

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 1494

<212> DNA

<213> Bacillus amyloliquefaciens

<400> 1

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gcacgatgag	cgccgctaag	aagaaagatg	tcattctga	aaaaggcggg	aaagtgc当地	300
agcaattcaa	atatgttagac	gcagcttcag	ctacattaaa	cgaaaaagct	gtaaaagaat	360
tgaaaaaaaga	cccgagcg	gttacgttg	aagaagatca	cgtagcacat	gcgtacgcgc	420
agtccgtgcc	ttacggcgta	tcacaaatta	aagccctgc	tctgcactt	caaggctaca	480
ctggatcaaa	tgttaaagta	gccccgttatcg	acagcggtat	cgattcttct	catctgtt	540
ttaaggtagc	aggcgagcc	agcatggttc	cttctgaaac	aaatccccc	caagacaaca	600
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gccaatacag	ctggatcatt	aacggaatcg	agtggcgat	cgcaaacaat	atggacgtt	780
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ccgttgcattc	cggtcgta	gtcgttgcgg	cagccgtaa	cgaaggact	tccggcagct	900
caagcacagt	gggctaccct	ggtaaatacc	cttctgtcat	tgca	gttgcgttgc	960
gcagcaacca	aagagcatct	ttctcaagcg	taggacctga	gcttgatgtc	atggcacctg	1020
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caaacactca	agtccgcagc	agtttagaaa	acaccactac	aaaacttgg	gattttct	1200
actatggaaa	agggctgtatc	aacgtacagg	cgccagctca	gtaaaacata	aaaaaccggc	1260
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cgatggctc	cctctgaaaa	tttaacgag	aaacggcggg	ttgacccggc	tcagtccgt	1380
aacggccaag	tcctgaaacg	tctcaatcgc	cgcttccgg	tttccggta	gctcaatgcc	1440
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<210> 2

<211> 382

<212> PRT

<213> Bacillus amyloliquefaciens

<220>
 <221> VARIANT
 <222> 163, 164
 <223> Xaa = Pro or Asn

 <221> VARIANT
 <222> 168
 <223> Xaa = Asp or Asn

 <221> VARIANT
 <222> 195, 196
 <223> Xaa = Ser or Ala

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 <222> 205, 206
 <223> Xaa = Asp or Ala

 <221> VARIANT
 <222> 265, 266
 <223> Xaa = Ser or Thr

 <221> VARIANT
 <222> 358
 <223> Xaa = Glu or Gln

 <400> 2
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 Ile Phe Thr Met Ala Phe Gly Ser Thr Ser Ser Ala Gly Ala Ala Gly
 20 25 30
 Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met
 35 40 45
 Ser Thr Met Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly
 50 55 60
 Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala Ala Ser Ala Thr
 65 70 75 80
 Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala
 85 90 95
 Tyr Val Glu Glu Asp His Val Ala His Ala Tyr Ala Gln Ser Val Pro
 100 105 110
 Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr
 115 120 125
 Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser
 130 135 140
 Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala Ser Met Val Pro Ser
 145 150 155 160
 Glu Thr Xaa Xaa Phe Gln Asp Xaa Asn Ser His Gly Thr His Val Ala
 165 170 175
 Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala
 180 185 190
 Pro Ser Xaa Xaa Leu Tyr Ala Val Lys Val Leu Gly Xaa Xaa Gly Ser
 195 200 205
 Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn
 210 215 220
 Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala
 225 230 235 240

Ala	Leu	Lys	Ala	Ala	Val	Asp	Lys	Ala	Val	Ala	Ser	Gly	Val	Val	Val
					245				250					255	
Val	Ala	Ala	Ala	Gly	Asn	Glu	Gly	Xaa	Xaa	Gly	Ser	Ser	Ser	Thr	Val
					260				265					270	
Gly	Tyr	Pro	Gly	Lys	Tyr	Pro	Ser	Val	Ile	Ala	Val	Gly	Ala	Val	Asp
					275				280					285	
Ser	Ser	Asn	Gln	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly	Pro	Glu	Leu	Asp
					290				295				300		
Val	Met	Ala	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr	Leu	Pro	Gly	Asn	Lys
					305				310				315		320
Tyr	Gly	Ala	Tyr	Asn	Gly	Thr	Ser	Met	Ala	Ser	Pro	His	Val	Ala	Gly
					325				330				335		
Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	Trp	Thr	Asn	Thr	Gln
					340				345				350		
Val	Arg	Ser	Ser	Leu	Xaa	Asn	Thr	Thr	Thr	Lys	Leu	Gly	Asp	Ser	Phe
					355				360				365		
Tyr	Tyr	Gly	Lys	Gly	Leu	Ile	Asn	Val	Gln	Ala	Ala	Gln			
					370				375				380		

<210> 3

<211> 275

<212> PRT

<213> Bacillus amyloliquefaciens

<400> 3

Ala	Gln	Ser	Val	Pro	Tyr	Gly	Val	Ser	Gln	Ile	Lys	Ala	Pro	Ala	Leu
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His	Ser	Gln	Gly	Tyr	Thr	Gly	Ser	Asn	Val	Lys	Val	Ala	Val	Ile	Asp
									20	25				30	
Ser	Gly	Ile	Asp	Ser	Ser	His	Pro	Asp	Leu	Lys	Val	Ala	Gly	Gly	Ala
									35	40				45	
Ser	Met	Val	Pro	Ser	Glu	Thr	Asn	Pro	Phe	Gln	Asp	Asn	Asn	Ser	His
									50	55				60	
Gly	Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly
					65				70	75				80	
Val	Leu	Gly	Val	Ala	Pro	Ser	Ala	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu
					85				90					95	
Gly	Ala	Asp	Gly	Ser	Gly	Gln	Tyr	Ser	Trp	Ile	Ile	Asn	Gly	Ile	Glu
					100				105				110		
Trp	Ala	Ile	Ala	Asn	Asn	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly
					115				120				125		
Pro	Ser	Gly	Ser	Ala	Ala	Leu	Lys	Ala	Ala	Val	Asp	Lys	Ala	Val	Ala
					130				135				140		
Ser	Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Glu	Gly	Thr	Ser	Gly
					145				150				155		160
Ser	Ser	Ser	Thr	Val	Gly	Tyr	Pro	Gly	Lys	Tyr	Pro	Ser	Val	Ile	Ala
					165				170				175		
Val	Gly	Ala	Val	Asp	Ser	Ser	Asn	Gln	Arg	Ala	Ser	Phe	Ser	Ser	Val
					180				185				190		
Gly	Pro	Glu	Leu	Asp	Val	Met	Ala	Pro	Gly	Val	Ser	Ile	Gln	Ser	Thr
					195				200				205		
Leu	Pro	Gly	Asn	Lys	Tyr	Gly	Ala	Tyr	Asn	Gly	Thr	Ser	Met	Ala	Ser
					210				215				220		
Pro	His	Val	Ala	Gly	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	
					225				230				235		240
Trp	Thr	Asn	Thr	Gln	Val	Arg	Ser	Ser	Leu	Glu	Asn	Thr	Thr	Thr	Lys
					245				250				255		

Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
260 265 270
Ala Ala Gln
275

<210> 4
<211> 275
<212> PRT
<213> Bacillus subtilis

<400> 4
Ala Gln Ser Val Pro Tyr Gly Ile Ser Gln Ile Lys Ala Pro Ala Leu
1 5 10 15
His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
20 25 30
Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala
35 40 45
Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His
50 55 60
Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly
65 70 75 80
Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
85 90 95
Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
100 105 110
Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
115 120 125
Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser
130 135 140
Ser Gly Ile Val Val Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly
145 150 155 160
Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala
165 170 175
Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala
180 185 190
Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
195 200 205
Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr
210 215 220
Pro His Val Ala Gly Ala Ala Leu Ile Leu Ser Lys His Pro Thr
225 230 235 240
Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr
245 250 255
Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
260 265 270
Ala Ala Gln
275

<210> 5
<211> 274
<212> PRT
<213> Bacillus licheniformis

<400> 5
Ala Gln Thr Val Pro Tyr Gly Ile Pro Leu Ile Lys Ala Asp Lys Val
1 5 10 15
Gln Ala Gln Gly Phe Lys Gly Ala Asn Val Lys Val Ala Val Leu Asp

	20	25	30
Thr	Gly Ile Gln Ala Ser His Pro Asp Leu Asn Val Val Gly Gly Ala		
	35	40	45
Ser	Phe Val Ala Gly Glu Ala Tyr Asn Thr Asp Gly Asn Gly His Gly		
	50	55	60
Thr	His Val Ala Gly Thr Val Ala Ala Leu Asp Asn Thr Thr Gly Val		
	65	70	75
			80
Leu	Gly Val Ala Pro Ser Val Ser Leu Tyr Ala Val Lys Val Leu Asn		
	85	90	95
Ser	Ser Gly Ser Gly Ser Tyr Ser Gly Ile Val Ser Gly Ile Glu Trp		
	100	105	110
Ala	Thr Thr Asn Gly Met Asp Val Ile Asn Met Ser Leu Gly Gly Ala		
	115	120	125
Ser	Gly Ser Thr Ala Met Lys Gln Ala Val Asp Asn Ala Tyr Ala Arg		
	130	135	140
Gly	Val Val Val Val Ala Ala Ala Gly Asn Ser Gly Asn Ser Gly Ser		
	145	150	155
			160
Thr	Asn Thr Ile Gly Tyr Pro Ala Lys Tyr Asp Ser Val Ile Ala Val		
	165	170	175
Gly	Ala Val Asp Ser Asn Ser Asn Arg Ala Ser Phe Ser Ser Val Gly		
	180	185	190
Ala	Glu Leu Glu Val Met Ala Pro Gly Ala Gly Val Tyr Ser Thr Tyr		
	195	200	205
Pro	Thr Asn Thr Tyr Ala Thr Leu Asn Gly Thr Ser Met Ala Ser Pro		
	210	215	220
His	Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn Leu		
	225	230	235
			240
Ser	Ala Ser Gln Val Arg Asn Arg Leu Ser Ser Thr Ala Thr Tyr Leu		
	245	250	255
Gly	Ser Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Glu Ala Ala		
	260	265	270
Ala	Gln		

<210> 6
 <211> 269
 <212> PRT
 <213> Bacillus lentinus

<400>	6			
Ala	Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala			
	1	5	10	15
His	Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp			
	20	25	30	
Thr	Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser			
	35	40	45	
Phe	Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr			
	50	55	60	
His	Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu			
	65	70	75	
			80	
Gly	Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala			
	85	90	95	
Ser	Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala			
	100	105	110	
Gly	Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser			
	115	120	125	
Pro	Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly			

130	135	140
Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser		
145	150	155
Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln		160
165	170	175
Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile		
180	185	190
Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr		
195	200	205
Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala		
210	215	220
Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile		
225	230	235
Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu		240
245	250	255
Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg		
260	265	

<210> 7
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 7
gtgtgtggc ccatcagtct gacgacc 27

<210> 8
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 8
gtgtgtggc cctattcgga tatttag 27

<210> 9
<211> 275
<212> PRT
<213> Artificial Sequence

<220>
<223> consensus sequence

<221> VARIANT
<222> (1)...(275)
<223> Xaa = Any Amino Acid

<400> 9
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His Xaa Xaa Gly Xaa Thr Gly Ser Xaa Val Lys Val Ala Val Xaa Asp
20 25 30

Xaa Gly Xaa Xaa Xaa Xaa His Pro Asp Leu Xaa Xaa Xaa Gly Gly Ala
35 40 45
Ser Xaa Val Pro Xaa Xaa Xaa Xaa Xaa Gln Asp Xaa Asn Xaa His
50 55 60
Gly Thr His Val Ala Gly Thr Xaa Ala Ala Leu Asn Asn Ser Ile Gly
65 70 75 80
Val Leu Gly Val Ala Pro Ser Ala Xaa Leu Tyr Ala Val Lys Val Leu
85 90 95
Gly Ala Xaa Gly Ser Gly Xaa Xaa Ser Xaa Leu Xaa Xaa Gly Xaa Glu
100 105 110
Trp Ala Xaa Asn Xaa Xaa Xaa Xaa Val Xaa Asn Xaa Ser Leu Gly Xaa
115 120 125
Pro Ser Xaa Ser Xaa Xaa Xaa Xaa Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa
130 135 140
Xaa Gly Val Xaa Val Val Ala Ala Xaa Gly Asn Xaa Gly Xaa Xaa Xaa
145 150 155 160
Xaa Xaa Xaa Xaa Xaa Tyr Pro Xaa Xaa Tyr Xaa Xaa Xaa Xaa Ala
165 170 175
Val Gly Ala Xaa Asp Xaa Xaa Asn Xaa Xaa Ala Ser Phe Ser Xaa Xaa
180 185 190
Gly Xaa Xaa Leu Asp Xaa Xaa Ala Pro Gly Val Xaa Xaa Gln Ser Thr
195 200 205
Xaa Pro Gly Xaa Xaa Tyr Xaa Xaa Xaa Asn Gly Thr Ser Met Ala Xaa
210 215 220
Pro His Val Ala Gly Ala Ala Ala Leu Xaa Xaa Xaa Lys Xaa Xaa Xaa
225 230 235 240
Trp Xaa Xaa Xaa Gln Xaa Arg Xaa Xaa Leu Xaa Asn Thr Xaa Xaa Xaa
245 250 255
Leu Gly Xaa Xaa Xaa Xaa Tyr Gly Xaa Gly Leu Xaa Asn Xaa Xaa Ala
260 265 270
Ala Xaa Xaa
275

<210> 10
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> assay protein

<400> 10
Ala Ala Pro Phe
1